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# Calcium, An Overview—1989

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**Abstract.** An overview of calcium is presented including introduction, pre-history, chronology of the research recorded in the literature, discussion, summary, recent references, literature cited, acknowledgments, and appendix.

Elemental calcium began with the Earth's formation. Calcium was used for utilitarian purposes in B.C. times. In the 12th and 13th centuries A.D., calcium oxide was formed by roasting limestone to form calcium carbonate. A test for calcium was found in the 17th century, and "stones" were observed in humans (see appendix). In the 19th century, calcium was isolated and chemically identified by electrolysis, and later in that century calcium was found to be needed in a physiological solution similar to the ionic content of blood. In the 20th century it was found that, in the absence of calcium, living cells pulled away from one another. Anesthesia was produced by massive injection of magnesium salts into a mammal—conciousness could be restored by the addition of calcium, which neutralized the magnesium. Finally, calcium out of control in necrosis has an invasive action. Calcium antagonists and their mode of action were described in 1986.

## Introduction

While a university fellow and research assistant to the late Dr. L. V. Heilbrunn, I developed a deep interest in calcium—an interest that has culminated in this manuscript after 48 summers at the Marine Biological Laboratory in Woods Hole, Massachusetts. This is my tribute to Dr. Heilbrunn for his research and profound vision of the importance of calcium in biological systems.

In 1983 the role of calcium dominated my conversations with Associate Professor Dr. Lincoln Ford of the University of Chicago. He urged me to write this account of the history of calcium.

The calcium ion is of fundamental importance in all biological systems. It participates in numerous biochemical and physiological processes. It is an intracellular regulator and also functions in extracellular mechanisms. For example, calcium is involved in neurotransmission, muscle contraction, blood clotting, cell adhesion, cell movement, permeability, and the surface precipitation reaction. It is also a second messenger, a major component of bone and teeth, and is important in agriculture and nutrition (Fig. 1).

Since calcium plays such a central and compelling role in all of basic biology, many books would have to be written in order to discuss all the phases and functions of calcium. Literature on the history of calcium dates back to 2600 B.C; the first practical problem—"stones"—was recorded in 1683 A.D. (see appendix).

Publications on calcium's importance, however, were sporadic and drew little attention until the Heilbrunn and Wiercinski paper (1948) describing microinjection of calcium into isolated muscle fibers. At that time, A. V. Hill questioned the slow diffusion of calcium into the cell. Niedergerke (1955) gave further impetus and brought attention to calcium with intracellular microinjection and electrolytic transport.

Since then, the interest in, and study of, calcium has increased dramatically. Approximately 22,000 papers have been published between 1966 and 1987 on the generation of concepts, hypotheses, mechanisms, models, postulates, and theories concerning calcium (Table I).

## Pre-History

The overview of calcium begins with the origin of the sun, the earth, and other planets formed by the condensation of interstellar gases, particles, and elements brought together by Newtonian forces and by thermal,

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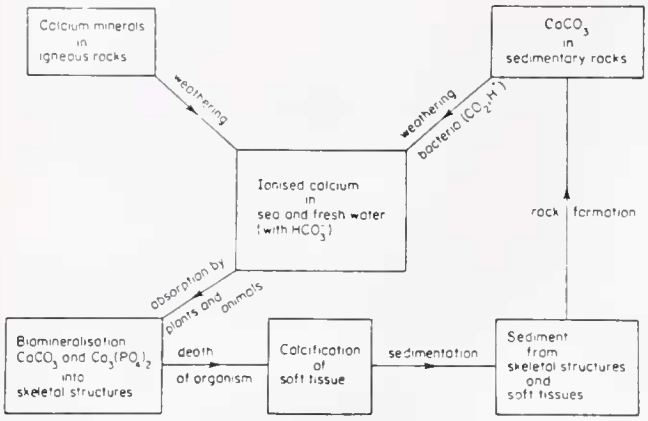


**Figure 1.** Calcium phosphate cycle in the biosphere. The relationship between plant and animal life to the land and sea cycle. (Reprinted with permission; from Nichols and Wasserman, 1971).

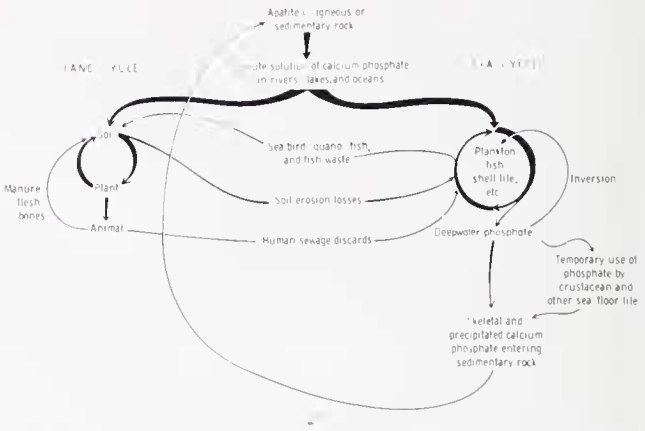


**Figure 2.** The biogeochemistry of calcium. The precipitates of calcium phosphate and carbonate are the major inorganic constituents of skeletal structures. In combination with collagen, rigid and strong structures are formed. (Reprinted with permission; from Campbell, 1983.)

electrostatic, and magnetic attractions at the time of the sun's formation. The earth went into orbit around the sun. Rock formed as the earth was pulled together by gravitational attraction. Consequently the planet grew in size. One major constituent of the new planet was calcium.

Water formed from hydrogen and oxygen. This filled the valleys and ocean basins of the earth. Water washed the rocks of the earth, leaching out elements including calcium into the ocean. Calcium is still being deposited into the oceans as sedimentary $CaCO_3$ from combination with carbon dioxide (Fig. 1). See Figure 2 for the biogeochemistry of calcium.

### *Ancient calcium*

"The calcium carbonate of the rivers would supply this substance in $5 \times 10^5$ years. This has been deposited as sedimentary limestone. Since the formation of the oceans, water must have carried to the dwellers of the sea not less than $3 \times 10^{17}$ tons . . . of calcium carbonate, which they have utilized as structural material" (Henderson, 1913).

If we take $5 \times 10^5$ years and divide it into $3 \times 10^{17}$ tons of calcium carbonate, we would have $6 \times 10^{11}$ tons per year. Forty percent of calcium carbonate is calcium. This is $6 \times 10^{11} \times 0.4$, which equals $2.4 \times 10^{11}$ tons of calcium per year. This was deposited as sedimentary limestone on ocean floors.

Ancient calcium, with other ions, has been added to the oceans for 100 million years. Data from Williams (1971; *loc. cit.*, Sillan) shows that calcium is the third ion in abundance after sodium and magnesium. The fourth most abundant ion is potassium. These ions are essential for living systems.

**Table I**

*Computer search (1966–1987) on concepts about calcium in the literature*

| | Agricola 1979–87 | Biosis 1981–87 | Life Sciences 1978–87 | Medline 1966–87 |
|---|---|---|---|---|
| Concepts | 12 | 167 | 127 | 577 |
| Hypotheses | 13 | 651 | 611 | 1,880 |
| Mechanisms | 49 | 3,393 | 2,392 | 7,728 |
| Models | 16 | 207 | 260 | 3,446 |
| Postulates | 0 | 4 | 3 | 16 |
| Theories | 1 | 16 | 15 | 65 |
| TOTAL | 91 | 4,438 | 3,408 | 13,712 |

Grand total: 21,649 papers over 21 years.

## Chronology

### *Early observations*

From earliest times, man recognized that something was necessary in the structure and function of bones, teeth, and blood. That it was calcium became known much later. The history of calcium spans the last five centuries. A list of the functions of the calcium ion in time, as well as the scientists involved in determining those functions, follows.

### *B.C.*

2600, Egypt: Gypsum ($CaSO_4$) mortar was used to create a seal between limestone blocks to preserve a boat in a sealed pit (Monastersky, 1987).

1336, Egypt: Alabaster, gypsum, used for vases, figurines, unguent jars, and chests.

Egypt: Plaster used to cover bandages used for wrapping mummies; the masks were made of plaster (Van Rens, 1987).

China: Limestone and mortar used in Guilin, a 2000-year-old city built during the Han Dynasty (Fang, 1987, pers. comm.).

Romans: Made plaster casts of body parts. Decorations in buildings and mortar (Van Rens, 1987).

1500, Ebers Papyrus: No mention of calcium. Sea salts were added to a mixture of herbs, made into a ball and inserted into the anus as a cure for diarrhea (Dawson, 1935).

400, Hippocrates: Used sounds to explore urinary bladder for calculi (in Berdoe, 1893).

*A.D.: 12th–13th century*

Limeburners: Reduction of limestone ($CaCO_3$) to lime (CaO).

*17th century*

1666, Boyle: White precipitate for calcium salts with sulfuric acid. Used as a test.

1666, Boyle: Aquafortis, Aquaregia, and Oyl of Vitriol. Used on various substances.

1667, Fairfax: Mr. Goodrich, surgeon at Bury St. Edmund's, found 96 small stones in the bladder of a lad. He also found a stone almost as large as a newborn child's head, and much of that shape.

1672, Lister: A stone was cut from under a man's tongue.

1683, 1693, Slare: A stone the size of a kidney was found. Stones also found in the vessels of the pelvis (see appendix).

*18th century*

1753, Black: Efficacy of limewater in dissolving urinary calculi, "the stone."

1786, Muller: "Sarcode droplets" on a *Colpoda* protozoan.

1794: Arabs used gypsum plaster casts (Van Rens, 1987).

1797, Wollaston: Chemical analysis of renal calculi.

*19th century*

1800, Volta: Voltaic pile battery.

1808, Davy: Calcium was isolated by electrolysis and the decomposition of alkalis.

1808, Davy: Metals obtained from alkaline earths.

1829, Dobereiner: Classified elements (see Discussion).

1835, 1841, Dujardin: Film formation around droplets emerging from a protozoan in calcium solution.

1846, Von Mohl: The name of "protoplasm" applied to the fluid within the cell.

1852, Muthijens: A new method of applying plaster bandages in the eare of broken bones (Van Rens, 1987).

1857, Haeckel: Crustacean nerve was pressed, forms of "droplets, thread, and granules" came out of the nerve.

1882, 1886, 1890, Ringer: Physiological salt solution for cell function as the external electrolyte.

1885, Ringer and Buxton: Distilled water is actually harmful to protoplasm. Minute quantities of salts, calcium, sodium, potassium obviate the destruction.

1886, 1892a,b, Gruber: Cutting experiments, *Stentor* and structure, *Infusoria*, all in calcium containing media.

1888, Fabre-Domergue: Film formation around droplets of protoplasm pushed out of cells.

1890, Ringer: Added calcium chloride to distilled water and life was sustained longer than in sodium and potassium salt solutions. Tap water worked because of the calcium in it, whereas distilled water did not.

1892, Loew: Calcium counteracts magnesium when the magnesium concentration is too high in plants.

1892, Loew: Nucleus contains calcium mainly in the chromatin and the nucleolus.

1894, Locke: Calcium necessary for the transmission of excitatory nerve impulses from nerve to muscle. A nerve-muscle preparation in calcium-free solution, the muscle failed to respond when nerve was stimulated. Addition of small amount of calcium restored excitation by nerve. This response depended on the presence of calcium in the solution.

1894, Ringer and Sainsbury: "Remarkable power with a minute dose of lime salt, *e.g.*, lime phosphate possesses in maintaining the integrity of tissues." "Whilst a very minute quantity of a lime salt serves to be adequate to the maintenance of this vitality (of the worm *Tubifex*), the quantity may be increased enormously without producing any very definite results."

1896, Biedermann: 0.6% NaCl was found to be better with $Na_2HPO_4$ and $Na_2CO_3$ (Vol. 1, p. 105).

*20th century*

1900, Herbst: Cells tend to separate in the absence of calcium.

1902, Kolsch: Film formation around droplets of protoplasm in calcium media.

1902, Loeb: Pure calcium solution is toxic to *Fundulus* eggs. Isotonic solutions of calcium are toxic to cells and organisms adapted to living in the presence of relatively high concentrations of salts.

1903, Stiles; 1912, Trendelenburg; 1920, Alday-Redonnet; 1926, Gellhorn: Smooth muscle is more permeable to calcium than skeletal muscle. A prompt shortening occurs.

1904, Overton: Shortening occurs in uninjured muscle. Calcium necessary for transmission of impulses across the nerve synapse.

1905, 1908, Meltzer and Auer: Injected magnesium salts into the blood stream of a vertebrate. A sufficiently high concentration produced a state of anesthesia. When calcium was injected, the animal became sensitive and awakened.

1902, Loeb: Calcium and other ions in irritability and contraction.

1907, Benecke: *Spirogyra* not injured by calcium alone.

1908, Daniel: The destructiveness of distilled water has been attributed to its low content of inorganic salts. Distilled water of low conductivity and of physical purity was obtained.

1909, Mendel and Benedict: Excretion of calcium and interrelationship to inorganic compounds.

1909, Osterhout: K and Na act antagonistically.

1910, Meyer; 1926, Gray; 1941, Robertson: Calcium important in holding cells together.

1911, Mines: Exclusion of calcium from Ringer's solution caused uncoupling of the action potential of heart muscle from contraction. This was the first evidence of the role of calcium in coupling excitation and contraction.

1913, Spaeth: K and Na act antagonistically. K is seven times more potent than Na.

1916, Peck and Meltzer: Surgical operations were performed with magnesium anesthesia en masse (see 1905–1908).

1918, Loewi, a, b: Calcium is necessary in the digitalis effect on the heart (p. 93). Calcium prevents digitalis standstill in the heart.

1920, Alday-Redonnet: Adrenalin and calcium.

1920, Hober; 1932, Gillespie and Thornton: All types of muscle fail to contract in the absence of calcium.

1921, Loewi: "Vagus-stoff" from vagus nerve was a fundamental discovery. In the course of events it was observed that calcium plays an important role in the secretion of acetylcholine (ACH) that is "vagus-stoff."

1922, Burridge: "The paralyzing action of cocaine must be associated with some form of deprivation of the effected tissues of their calcium."

1922, Tate and Clark: Calcium chloride produces contraction of rabbit and cat uteri; relaxation of in guinea pig and rat uteri.

1923, Robison: Advance concerning conversion of blood calcium into the insoluble calcium of bone.

1925, 1931, Loew; 1926, 1928, Pringsheim: All animals from amoeba to man and all plants except some lower algae and fungi require calcium. A few yeasts and molds do not require calcium.

1926, Chambers and Reznikoff: Studied the actions of monovalent and divalent ions on amoebae using immersion experiments and microinjection.

1927, Doflein and Reichenow: Cutting experiments of Protozoa in calcium solution.

1927, Bouckaert and Colle: Magnitude of muscle contraction is a function of the amount of calcium present.

1927, Fitting: Calcium antagonizes the effect of poisons.

1927, Gley and Bouckaert; 1928, Gley; 1928, Houssay and Molinelli; 1929, Titajew and Unik: When calcium is lacking, nerve fibers, especially fibers of the autonomic system, fail to respond to stimulation.

1927, Heilbrunn: "Surface precipitation reaction." When *Stentor* was crushed in a calcium medium, a new membrane formed around the extruded cell protoplasm.

1928, Guissani; 1939, Beutner: Convulsions in dogs by injection of cocaine can be prevented by simultaneous (injection) action of calcium chloride.

1928, Kapeller and Kutschera-Aichbergen: Calcium is necessary for heart muscle function.

1928, Lawaczek: Adrenaline seems to release calcium from the bound to the free state.

1928, Pollack: By microinjection of calcium, precipitating agents showed the relationship between intracellular calcium and cell function such as movement.

1928, Rubenstein: Diagram for antagonism of cations (p. 408, Heilbrunn, 3rd edition). K and Na are mutually antagonistic. Ca and Mg antagonize Na but are synergistic.

1928, Simon and Szeloczey: Cocaine causes release of calcium from tissues.

1929, McCutcheon and Lucke: Both calcium and magnesium induced permeability compared to sodium and potassium.

1930, Chambers and Howland: Injections of $CaCl_2$, produce a dense granular coagulum in amoeba.

1930, Heilbrunn: High calcium concentration prevents protoplasmic clotting. Calcium is necessary for the surface precipitation reaction; higher concentrations of calcium ion have the same effect as other ions in retarding or inhibiting it.

1930, Pauling and Goudsmit: The level scheme of calcium, the metastable state.

1931, Camac: Medical anatomy from Imhoteps to Harvey.

1931, Kisch: An ion that is antagonistic to a second ion at one concentration may act with it at another concentration. According to Kisch, this relation exists between Mg and Ca.

1931 Kisch; 1934, Lasnitzki: Calcium lack may cause a decrease in the rate of respiration.

1932, Gillespie and Thornton: Isolated bronchial muscle of guinea pigs is sensitive to changes as low as 1

mgm 0/0 ionic concentration of calcium as measured by the response to histamine (p. 425).

1932, Heilbrunn: Magnesium and potassium anesthesia in amoeba. The colloid chemistry of protoplasm.

1934. Bacq and Rosenbleuth; 1936, Hukuda and Moriya: Heart muscle stops in a contracted state (systole) with calcium.

1938, Gaede: The working of the alkaline salts.

*Antagonism of cations*

1932, Costello: Calcium is a necessary requisite for the surface precipitation reaction in marine eggs.

1932, Heilbrunn: Simplest type of antagonist depends on the valence of the cation (p. 467). In sea urchin eggs, both the Mg and Ca tend to prevent the toxic effects of monovalent cations Na and K.

1932, Hermann: Calcium is necessary for the production of adrenaline.

1933, Breder: Marine fish can withstand fresh water when it contains a small amount of calcium.

1933, Ellis: Calcium and the resistance of *Nereis* to brackish water.

1934, Hastings *et al.:* The behavior of calcium in the presence of citrate, holding calcium in a non-ionizable form, in regard to the state of calcium in the living organism.

1934, Heilbrunn: The effect of anesthetics on the surface precipitation reaction. At low calcium concentration, ether inhibits the surface precipitation reaction in *Arbacia* eggs.

1934, McLean and Hastings: Frog heart used to test quantitatively the presence of definite amounts of calcium ion. Perhaps the best physiological example of reversible binding of calcium ion to serum proteins.

1934, Peters *et al.:* When calcium is added to normal uninjured mammalian heart tissue, it may cause an increase in the rate of respiration. At a given initial fiber length, the mechanical energy of the heart is increased under the influence of calcium.

1935, Buchanan: Very dilute solution of calcium salts protect planaria from the effects of distilled water.

1935, Dawson: Healing pagan and Christian (see 1500 B.C., Ebers Papyrus). Egyptian physicians used seawater in a suppository.

1935, Huf: Concept of active transport.

1936, Keil and Sichel: Injection of small quatities of $CaCl_2$ into isolated frog muscle M/200 caused violent but reversible shortening. (No data on quantity given.)

1936, Wiercinski and Child: Differential susceptibility of living organisms to supersonic vibration in calcium media.

1937, Ellis: Calcium plays a role in the exchange of water and electrolytes in *Nereis diversicolor.*

1937, Marcozzi; 1939, Mast and Pace: All commercial preparations of magnesium salts contain calcium.

1937, von Euler: Calcium chloride in skeletal and heart muscle studies in concentrations of 0.1 mol/l is necessary.

1937, von Euler; 1939, Greville: Respiration of minced muscle is decreased by calcium.

1937, Heilbrunn and Wilbur: Sodium citrate in seawater inhibited the germinal vescicle breakdown of *Nereis limbata* eggs induced by agents such as ultraviolet light or hypertonic potassium chloride in seawater. The normal response of the egg was restored by adding $Ca^{2+}$.

1937, Mazia: Release of $Ca^{2+}$ was found in homogenates of fertilized *Arbacia punctulata* eggs.

1938, Ashkenaz: Narcotizing action of magnesium for whole muscle and single fibers can be antagonized by adding calcium ions to the medium. Recovery is best in Ringer's solution or in Ca-Mg-Ringer's solution containing four times the calcium content of ordinary Ringer's.

1938, Eggleton *et al.:* Calcium considered in 13 citations on all phases of heart action (frog) (see references).

1938, Goreczky and Ludany: In man, injection of excess calcium into the blood stream lowers the basal metabolic rate.

1938, Mast and Fowler: Sodium, potassium, and calcium ions in relation to volume changes in *Amoeba proteus.*

1939, Alexander *et al.:* Ca cephalinate is relatively insoluble compared with Na or K cepalinate in a liquid/liquid interface.

1939, Graham and Sichel: Local application of $CaCl_2$ to the surface of isolated skeletal muscle fiber caused reversible shortening of the muscle substance.

1939, Henry and Kon: In all comparisons, milk Ca was more available than the Ca of $CaHPO_4$, $2H_2O$.

1939, Mollison: Calcium deficiency lowers rate of respiration in plants—especially of the tops.

1939, Wiercinski: The effect of supersonic vibration on reconstitution and head frequency in *Euplanaria dorotocephala* in calcium medium.

1940, Guttman: Calcium maintained the resting membrane potential from other effects such as high potassium.

1940, Harvey and MacIntosh: In perfusion fluid lacking calcium, the release of acetylcholine was diminished or abolished. The effect of calcium was on the release reaction.

1941, Axelrod *et al.:* Calcium had a marked effect on the cytochrome-succinic-dehydrogenase system.

1941, Glick: Cholinesterase activity increased by calcium.

1941, Heilbrunn and Ashkenaz: The irritability of the isolated (cut) fiber is dependent on the calcium ion.

1941, Heilbrunn and Ashkenaz: Calcium rather than

sodium (Overton, 1904) appears to be the ion essential for muscular contraction as demonstrated with isolated muscle fibers.

1941, McDonald and Kunitz: Calcium markedly increased the proteolytic activity of pancreatic juice. The effect results from the activation of trypsinogen to trypsin.

1941, Wiercinski: Intracellular pH determination in the centrifuged hyaloplasm of the *Arbacia* egg in seawater.

1942, Bailey: Calcium is a potent activator of isolated myosin ATPase.

1942, Bailey: Calcium influences the activity of lipases. Calcium activates the enzymatic activity of myosin.

1942, Elvehjem: Mechanism in enzyme system may be an indirect one (see Swingle *et al.*, 1942).

1942, Swingle *et al.*: Calcium stimulates activity of succinoxidase, which oxidizes succinic acid.

1943, Heilbrunn: Calcium, magnesium, and other cations have very marked effects on protoplasm. To some extent these actions may be due to their action on enzyme systems. (p. 201)

1943, Heilbrunn: There is also an interesting analogy in the behavior of protoplasm toward calcium. (p. 466)

1943, Heilbrunn: Magnesium narcosis and reversal to a conscious rabbit by injection of calcium. (p. 461)

1943, Heilbrunn: Researchers used improper concentrations of magnesium and calcium salts in their experiments; these were not isotonic. (p. 464)

1943, Heilbrunn: When a frog nerve is placed in isotonic calcium chloride solution, a wave of death proceeds from the cut end. This wave travels at a rate of a third of a millimeter per minute (unpub. experiments of M.A. Robbins, see p. 466, footnote).

1943, Heilbrunn: Some effects ascribed to calcium are due to the hypertonicity of the calcium solution.

1943, Heilbrunn: "In sea urchin eggs a trace of calcium will protect the cells from the toxic action of sodium, and a further increase in calcium concentration has but little obvious effect" (p. 463).

1943, Heilbrunn: "The fact that these and other drugs affect the cell by way of its calcium is but another indication of the importance of calcium in the vital machinery" (p. 467).

1943, Heilbrunn: Cocaine, like many other anesthetics, can also at times show a stimulating action (Ch. 36, 37). Both as an anesthetic and a stimulant, cocaine is antagonized by calcium. (p. 467)

1943, Heilbrunn (from Ringer and Buxton, 1885): Cilia of a freshwater clam gill could beat for eight days in a pure calcium solution.

1943, Heilbrunn (from Ringer, 1890): Frog eggs can develop normally for days in a calcium phosphate solution.

1944, Wiercinski: Intracellular pH determination of hyaline protoplasm in centrifuged *Arbacia* eggs in seawater. Cytoplasmic pH in *Amoeba* proteus in calcium media.

1945, Hober: In the series of Na, K, Ca, and Mg, Ca is high in hydration.

1947, Heilbrunn and Wiercinski: Solutions of sodium, potassium, magnesium, and calcium chlorides were microinjected into isolated frog muscle fibers in calcium-free media. Calcium ion, in high dilution (M/5000) caused an immediate and pronounced shortening. Injectant was 10% of the muscle fiber volume. The fibers were dissected in calcium-free media.

1950, Terry; 1954, Gross; 1968, Bianchi: Cytoplasmic inclusions swell and rapidly dissolve in low calcium concentrations.

1952, Castillo and Stark: End plate potential may reach about three times its normal size due to high calcium.

1952, Engback: Magnesium and calcium ions affect neuromuscular transmission. Calcium ion acts as an activator.

1952, Wiercinski: Microinjection of M/5000 $CaCl_2$ into the isolated frog muscle fiber induces protoplasmic shortening at alkaline and acidic pHs.

1954, Gross: Calcium can disrupt cytoplasmic granules.

*Pharmacology*

1918a,b, Loewi; 1937, Gold and Kwit: Digitalis and similar compounds act on the vertebrate heart only if calcium is present.

1942, Otis; 1964, Hammond: Relation of digitalis and adrenaline effects to calcium in oyster heart.

1951, Heilbrunn: Calcium is not only involved in bones and teeth, but also in the vital mechanisms of soft protoplasm.

1956, Heilbrunn: Coagulative properties of calcium on lower organisms and the relation to blood clotting.

1956, Heilbrunn: Coagulative properties and stimulating properties of calcium.

1958, 1966, Katz: Calcium functions in the amphibian neuromuscular junction to release acetylcholine during depolarization by nerve impulses.

1958, Shanes: Calcium maintained nerve conduction in spite of the influence of other agents to alter the resting potential.

1965, Pease *et al.*: Electron microscopic localization of calcium uptake in glycerol extracted psoas muscle fibers.

1965, Ratnoff: Hemorrhagic disorders: coagulation defects and calcium (in Beeson and McDermott, 1975).

1966, Kliner and Orten: Lactose and vitamin D increase calcium and strontium absorption.

1967, Federation Meetings: pH and calcium in muscle.

1968, Douglas: Calcium is a general mediator of secretory mechanisms (see Rubin, 1982, recent references).

1968, Katz and Miledi: Facilitation is larger during the second impulse, in accordance with the calcium hypothesis.

1968, Langer: Calcium and ion fluxes in myocardial contractility.

1969, Carriker and Smith: Comparative calcibiocavitology.

1969, Carriker *et al.:* Calcium carbonate substrates.

1969, Jahn and Bovie: Cellular cytoplasmic movements in cells. Protoplasmic motility involved in an actomyosin protein complex triggered by calcium to split high energy phosphates.

1970, Ford and Podolsky: Regenerative calcium release in muscle cells.

1970, Podolsky and Teicholz: Comparison of calcium contraction kinetics in skinned and intact muscle fibers.

1971, Williams: The metals of life. Importance of calcium.

1973, Goodenough and Stenger: The name calcium comes from *calix*, the Roman word for lime.

1973, Swanson *et al.:* Saliva, calcium, and potassium concentrations in the detection of digitalis toxicity.

1974, Esmon and Jackson: The function of fragment 2 during presence of factor V in blood clotting.

1974, Rubin: Speculation on calcium actin. (Rubin, 1982, recent references)

*Enzymes*

1905a,b,c,d, Delezenne: Dilute solutions of calcium salts favored activation of the enzyme; more concentrated solutions had the opposite effect. Analogy is made to blood. Dilute calcium solutions favor clotting, more concentrated solutions prevent it.

1937, Eagle; 1940, Ferguson; 1940, Brinkhous; 1940, Wohlisch: Modern theories of blood clotting emphasize the important role that a proteolytic enzyme plays in the process involving calcium.

1966/67, Fleckenstein *et al.:* Coined the term "calcium antagonist" based on observations of verapamil and prenylamine to interfere with the mediator function of $Ca^{++}$ in the excitation-contraction coupling of heart muscle.

1969, Godfraind and Kaba: The term "calcium antagonist" was suggested because of the action of cinnarazine and chlorpromazine to inhibit the entry of calcium into depolarized smooth muscle cells.

1975, Arnold: Calcium causes contraction of the cleavage furrow in squid embryo cells. Embryos in calcium-free seawater will not cleave; an exaggerated cleavage of the dividing cells occurs in 2× calcium seawater.

1975, Palade: Secretion, intracellular aspects of protein secretion.

1976, Considine (ed.): Van Nostrand's Scientific Encyclopedia, 5th ed., "Calcium."

1976, Leaf and Cotran: Calcium, magnesium, and phosphate in pathorenal physiology.

1977, Suttie and Jackson: Prothrombin.

1977, Fleckenstein: Calcium antagonism in heart and smooth muscle. Calcium channels blocked by organic calcium antagonists *e.g.*, verapamil and nephedipine, oxyverapamil (D-600), and prenylamine.

1978, Kretsinger: Calmodulin.

1979, Kretsinger: Calcium in neurobiology. A general theory of its function and application. Neurobiology Manual, MBL.

1979: Symposia Soc. Exp. Biol. N. XXXIII, pp. 165–192. Conclusion: calcium ion plays a nearly universal role in all cell activities. Ca ion and cAMP are second messengers.

1980, Cheung: Calcium binding proteins as calcium receptors have been extended to a broad range of cellular reactions and processes. Calmodulin plays a pivotal role in cellular regulation.

1980, Ebashi: Szent-Györgyi's actomyosin-ATP-K system, which did not require calcium for activation, suppressed the calcium concept. Also, Heilbrunn did not appreciate the role of ATP.

1980: *Harvard Medical School Health Letter,* Vol. 5, No. 7, p. 5. The final common pathway for all triggers that initiate spasm may be an increase in the calcium flow into the smooth muscle cells lining the coronary arteries.

1981, Slater: Calmodulin may help explain the mechanism by which calcium regulates vital intracellular activities.

1982, Anghileri and Anghileri: Biological systems and calcium.

1982, Chafouleas *et al.:* Cell division—calmodulin and the cell cycle.

1982a,b, Jaffe: Possibility of methylation of calcium receptors and the genome to induce reversion is an earlier embryonic state for carcinogenesis (p. 306).

1982, Nuccitelli and Deamer: When pH is decreased in crayfish slow muscle fibers, the delayed outward $K^+$ current conductance decreases, allowing the inward $Ca^{2+}$ current to become regenerative, producing all-or-none action potentials in these normally relatively inexcitable cells. (p. 435)

1982, Rubin: *Calcium and Cellular Secretion.*

1982, Thomas: *Techniques in Calcium Research.*

1983, Durrier and Meijler: Calcium paradox, a de-

struction of the ultra- and microstructure of the myocardium, in ischaemic or perfusion damage.

1983, Campbell: Calcium and cell movement (pp. 206–256). Endocytosis and exocytosis: uptake and release of substances from cells (pp. 305–361). Intracellular $Ca^{2+}$ may play a key role in mediating the effects of primary stimuli on exocytosis, endocytosis, fluid secretion, and possibly steroidogenesis. The rationalization of these phenomena and their evolutionary significance has to be made (p. 361).

1983, Jaffe: Sources of calcium in egg activation.

1983, Morton: An annotated checklist of text illustrating the history of medicine. Urinary calculi (pp. 572–589).

1983, Spiro: Calcium signal transmitted through the interactions with target enzymes and structural proteins.

1983, Tsien: Calcium channels in cardiac muscle are hormone responsive.

1983: Calcium Theme, 67th Annual Meeting of the Federation of American Societies for Experimental Biology and Guest Societies. Chicago, IL. April 10–15, 1983.

1983: *Federation Proceedings.* Calcium, phosphorus, and related elements. Nutrition.

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## Discussion

### *Importance of calcium*

Calcium has appeared in the literature in one form or another since 2400 B.C. It is now known to be important in the external movement of cells, tissues, and organisms, in the intracellular movement of organelles, and in the formation of by-products released from cells.

Total understanding of the intra- and extracellular functional aspects of calcium must be considered using a multifaceted approach. Several authors have recently examined aspects of calcium's role in biological systems. Rubin *et al.* (1985) considered the modern historical and biological aspects of calcium action dating back to the time of Sydney Ringer (1882–1894). Schwartz and Azar (1981) described intracellular regulation and transport leading to calcium's control of physiological processes, and Campbell (1983) described intracellular calcium as the universal regulator.

We now know that eukaryotic cells maintain free $Ca^{2+}$ within the cytosol at a concentration between $10^{-7}$ and $10^{-8}$ *M* (Kretsinger, 1980). In muscle fibers, calcium is stored in the sarcoplasmic reticulum (Costantin *et al.*, 1965). Calcium's role in the formation of calculi in the kidney, gall bladder, and joints, as well as in the formation and structure of bones and teeth, are well documented.

### *Calcium's biological roles*

Calcium plays a number of important biological roles (Rubin, 1974, 1982; Schwarz and Azar, 1981; Anghileri and Anghileri, 1982; Campbell, 1983; Ebashi, 1984; Carafoli and Penniston, 1985; Rubin *et al.*, 1985).

Structurally, calcium is involved with second messengers, neurotransmitters, biomembranes, mechanical stress and muscle proteins, and cell-to-cell communication. Chemically, it is involved in the release of hormones. It is required for the maximum activation and regulation of enzymes and proteins, and it also serves as an intracellular regulator in association with calmodulin and hormone cyclic nucleotides. Calcium helps to maintain osmotic balance within cells by modulating cations, anions, and water permeability. It is also involved, in a variety of ways, in oxidation-reduction. Calcium's roles in tissue culture cells are also diverse: it is involved in adhesion, spreading, locomotion, cytokinesis, shape changes, and the closing of intracellular junctions. Finally, calcium plays important roles in cell motility (ruffling, blebbing, locomotion, displacement of mass, migration, and chemotaxis of leucocytes).

### *Isolation of calcium*

Davy began work on electrolysis after Volta's battery appeared in 1800. He isolated components of alkali salts dissolved in water. In 1808 he announced the isolation of potassium and sodium as mutants. Using similar methods, he isolated barium, magnesium, calcium, and strontium (Leicester, 1956).

Dobereiner (1829) then classified elements and found "triads" with similar properties. Calcium, strontium, and barium was one such triad, the atomic weight of the middle member being approximately the arithmetic mean of the weights of the other two elements.

### *Level scheme of calcium*

The lowest energy state of calcium occurs when the two outer electrons are both in the 4s orbit. When the single level occurs, the triplet is non-existent. In calcium's excited state, only one of the two outer electrons is excited, the other remains in the 4s state. The triplet levels are the so-called "metastable states." Via collision with other atoms, a calcium ion can drop to its "normal" state or the atom can absorb radiation to a state of higher excitation. It can then drop to the lowest level with the emission of radiation (Russell, 1927; Pauling and Goudsmit, 1930).

### *Plaster of Paris*

The ancient Egyptians and Romans applied techniques of using plaster of Paris as it is known today. The plaster bandage is hard setting and made of such components as gypsum ($CaSO_4$), starch, albumen, lead-vinegar, and spirits of camphor (van Rens, 1987).

Gypsum was used by the Arabs in 1794 in the treatment of fractures. They placed the fractured limb in a mold and poured gypsum over the broken extremity (van Rens, 1987).

In the 19th century, the use of plaster bandages was not easily accepted in Europe. The Thomas-urgoden splint was favored in England for quite some time.

### *The "stone"*

Calculi (stones) can occur in any hollow organ in the body in which its fluid content is stagnant. Stones have been known since ancient times. Descriptions of them have been found in ancient Summarian (pre-Babylonian) and early Egyptian writings. Dissection of mummies more than 5000 years old have revealed calculi, and in 900 A.D., the Arabians devised an operation for the removal of bladder stones (Benton, 1972).

In the 17th century, Boyle (1666) noted the formation of calcium when a calcite precipitate formed in sulfuric acid. Later, Fairfax (1667) and Slare (1683, 1693) reported "stones" in humans (see appendix). Black (1753) tried using limewater to dissolve urinary calculi. In 1797, Wollaston found renal calculi consisting of a mixture of

uric acid, calcium, magnesium, ammonium phosphate, and calcium oxalate. Nearly two centuries later, Thomsen (1986) used laser beams to disintegrate kidney stones. In 1989, extracorporeal shock wave lithotripsy shatters kidney and biliary stones. The body is immersed in water (*Health Scene,* 1989).

*Hypotheses generation*

A number of concepts, hypotheses, mechanisms, models, postulates, and theories have been generated regarding calcium since the 1st century A.D.

Venantius Fortunatus (600 A.D.) used the Latin word "protoplasma," meaning "first created thing, protoplast" (in Greek meaning "a moulded thing, figure, or form"). Müller (1786) described "droplets" in the protozoan *Colpoda.* This was an extrusion of the cell content into the surrounding water.

Dujardin (1835) postulated the concept of a "sarcode," an elementary substance composing the protozoan. Dujardin (1841) observed film formation of cell cytoplasm emerging from *Leucophrys striata,* a protozoan parasite found in the earthworm. Von Mohl (1846) named the sarcode "protoplasm." Huxley (1868) considered protoplasm "the physical basis of life."

Fabre-Domerque (1888) and Kolsch (1902) observed film formation around droplets of extruded protoplasm from a cell.

Heilbrunn (1930) postulated the "surface precipitation reaction." When the cell cortex is broken, the emerging protoplasm forms a membrane to seal the break in the presence of calcium ion. When cells are immersed in calcium-free media, the protoplasm forms vacuoles and flows outward from the injured cell.

Calcium is associated with a receptor protein in cells. In the "bleeders disease" where fibrinogen is inadequate, the blood does not clot because calcium, although present, has no fibrinogen to produce the fibrin required for blood clotting.

It is evident that Ca needs an "acceptor protein" in the cell. Heilbrunn (1928) suggested ovalbumen for the *Arbacia* egg; Kretsinger (1978), a protein in the cytosol; and Chance (1965), formation of an intermediate $Ca_2$ ($\sim$1) with the displacement of two protons—perhaps a metastable calcium as suggested by Pauling and Goudsmit (1930). Cheung (1980) suggested calmodulin, and others suggested leotonin, protein kinase, and protein kinase C.

In summary:

Heilbrunn (1930) postulated the role of calcium in the cell, and the "surface precipitation reaction."

Heilbrunn (1951) wrote that calcium plays many roles in the vital life processes such as cell division, aging, surface precipitation reaction, clotting of blood and cells, muscle contraction, and in a variety of ailments—from acne to the hardening of arteries.

Gross (1954) discussed the mechanisms for the yolk lysis reaction.

Chance (1965) wrote about the accumulation of calcium.

Fleckenstein (1977) discussed calcium antagonists such as verapamil.

Kretsinger (1979) wrote on the theory and evolution of calcium in neurobiology.

Cheung (1980) described calmodulin as playing a pivotal role on calcium.

Ebashi (1980) wrote that in 1960 there was considerable doubt about the "calcium hypothesis."

Campbell (1983) asked: does a stimulus activate 50% of the maximum possible "switched on" cells, or do all the cells give 50% of their individual response?

Putney (1986) reviewed hypotheses on the mechanism of calcium entry into the cell. He also wrote about the calcium pump.

Peterson (1987) discussed larger molecules, such as proteins and enveloping smaller molecules.

*Physiological solutions*

Ringer (1882, 1886, 1890) recognized the need for a physiological salt solution similar to the ionic content of blood. When calcium chloride was added to distilled water, life was sustained longer than in sodium and potassium solutions.

Ringer and Sainsbury (1894) found that minute doses of lime salt were necessary for the survival of the worm *Tubifex.*

Locke (1893, 1894) found that calcium was necessary for the transmission of nerve impulses from nerve to muscle.

Loeb (1906) wrote that life phenomena, and especially irritability, depend "on the presence in the tissues of a number of various metal proteids, or soaps (Na, Ca, K, and Mg) in definite proportions." Na-salts cause rhythmical contractions in striated muscle only if the muscle cells contain Ca-ions. When there is a lack of Ca-ions in the tissues, the Na-ions are no longer able to cause rhythmical contractions. Because plants contain no muscle, there is no need for NaCl.

*Anesthesia*

Meltzer and Auer (1905–1908) observed anesthesia in a rabbit following injection of a solution of magnesium. Upon injection of a small amount of calcium solution, the rabbit awakened and was sensitive. Peck and Meltzer (1916) performed surgical operations in humans, using magnesium anesthesia. Dogs will also go into anesthesia with magnesium salts (Wiercinski, pers. obs.).

*Active transport*

Huff (1935) suggested the concept of "active transport." Huff recognized the relationship of cellular metabolism and the role of extracellular metabolites to be transported into the living cell and the transport of substances out of the cell. The essential criteria are based on the following conditions in the living cell: (1) a concentrated and hydrated product; (2) sensitivity to oxygen lack; (3) dependence on active metabolites; and (4) sensitivity to enzyme poisons.

LeFevre presented work on the human red cell. Prior to his 1955 monograph, he wrote about the penetration of glycerol (1946), active transfer (1947), certain nonelectrolytes (1948), active transport of monosaccharides (1954), and active transport through cell membranes (1954).

Bresnick and Schwartz (1968, pp. 305–362) discussed active transport: methods of study, theories, drugs, hormones, and membranes.

The active transport concept was accepted from 1955 onward. However, the term "active transport" was used in different ways by various authors (see Giese, 1973, p. 324). The relationship of sodium channels and calcium in active transport is currently (1987) being studied by Clay Armstrong at the Marine Biological Laboratory (Woods Hole, Massachusetts).

*Frog heart study*

Clark (1938) describes the following in the frog heart:

(1) Duration of electrical and mechanical responses.
(2) Excess, refractory period of the heart and calcium.
(3) Heart's content of calcium.
(4) Hypodynamic heart and calcium.
(5) Lack, carbohydrate balance of heart with calcium.
(6) Lack, electrical response of heart and calcium.
(7) Lack, electrical response and metabolism.
(8) Lack, heart arrest and phosphagen with calcium.
(9) Lack, heart metabolism and action of calcium.
(10) Lack, mechanical response of heart and calcium.
(11) Loss of calcium in the heart on perfusion.
(12) Plasma and content of calcium.
(13) Sympathetic stimulation and liberation of calcium.

*Electrical properties*

Living cells have an "electrical property." Calcium reacts with the isolated muscle fiber, as Niedergerke (1955) demonstrated with calcium and electrolytic transport in local contraction and partial relaxation. The calcium ions activate a link in the contractile cycle. An electrical stimulus causes contraction. Presumably, when it is subthreshold, contraction is not elicited.

*Microinjection of freshly dissected muscle fibers*

It is recognized as a primary principle in the study of living organisms that the "living state" is of the utmost importance.

Hober (1920) and Gillespie and Thornton (1932) found that all types of muscle lose the ability to contract in the absence of calcium.

Keil and Sichel (1936) microinjected an aqueous solution of M/200 $CaCl_2$ and observed reversible contraction. No quantity was stated.

Graham and Sichel (1939) applied $CaCl_2$ to a small portion of an isolated muscle fiber surface and observed a marked reversible shortening of the "muscle substance."

Heilbrunn and Wiercinski (1947), in an extensive work to prevent contamination of solutions, used quartz distilled water from previously distilled water to prepare the solution to be microinjected. Quartz micropipettes were prepared for microinjection. These were "flamed" and reused. Pyrex micropipettes were used only once. Calcium ion at M/5000 caused a 50% "shortening" of the muscle fiber as compared to sodium and potassium ions in isotonic concentrations. Magnesium ion caused an extension of the muscle fiber (about 10% of the fiber volume).

Niedergerke (1955) microinjected muscle fibers with calcium and electrolytic transport and saw contraction in a reversible state.

Caldwell and Walster (1963) injected caffeine into crab muscle fibers. A contraction (contracture) was followed by a relaxation. The caffeine released the calcium from sarcoplasmic reticulum.

*Mechanism of muscle contraction*

Mines (1911) gave the first evidence for the role of calcium between excitation and contraction in muscle.

Fanburg and Gergely (1965) reported that the mechanism for the relaxation and contraction of skeletal muscle may operate in heart muscle. Calcium accumulation for skeletal and cardiac cells is optimal when ATP, magnesium, and oxalate are present in the *in vitro* preparations of sarcoplasmic reticulum and mitochondria.

*Mechanisms of cooperativity*

Cell components must cooperate to maintain cell function. Calcium binding to cell organelles and molecules depends on concentration and affinity for proteins in the population of $Na^+$, $K^+$, $Mg^{2+}$, $Cl^-$, $SO_2^{-2}$, $PO_4^{-3}$, etc. According to Pauling and Goudsmit (1929), calcium exists in a metastable state. This would involve electrons in cellular reactions (Peterson, 1987). Larger molecular mechanisms in cells can join with smaller molecules to

perform a variety of chemical functions in biological systems.

The most important mechanism of structure and function is cooperativity. Malphighi's discovery of the capillaries at least 200 years ago has recently been researched by Crone (1987) using the passage of solutions through capillary pores. Calcium may be an operative factor in the endothelial cells of the "endothelium derived relaxing factor" (EDRF).

*Accumulation of calcium (based on Bianchi, 1968)*

Chance (1965) postulated that accumulation of calcium can occur in the following steps (steps 5 and 6 are alternative mechanisms):

(1) Passive diffusion of calcium through the outer membrane of mitochondria (in the cytosol) to binding sites on the inner membrane;

(2) Formation of an intermediate of $Ca_2$ ($\sim$1) with the displacement of two protons;

(3) Transport of the energy-rich intermediate complex across the inner wall of the mitochondria;

(4) Binding of calcium to a phospholipid component of the inner membrane leading to the release of the components of the intermediate complex and the stimulation of respiration;

(5) Formation of hydroxyapatite or calcium phosphate crystals, thereby removing calcium from the mitochondrial membrane wall and allowing further re-accumulation of calcium;

(6) Neutralization of the liberated protons by carboxylic acids and mitochondria swelling.

*Trends*

Loewi (1912, 1918a,b) found that calcium is necessary for the digitalis effect on the heart. He also performed the classical "vagus-stoff" experiment (1921). Later it was found that the active principle is acetylcholine. Calcium is necessary for this reaction.

Chambers *et al.* (1926, 1930, 1932) did studies using micromanipulation on amoeba, starfish, and *Arbacia* eggs.

Keil and Sichel (1936) observed the effect of injecting M/200 $Ca^{2+}$ ion concentration in isolated muscle fibers. They did not determine injection volume.

Heilbrunn and Wiercinski (1948) microinjected $Na^+$, $K^+$, $Mg^{2+}$, and $Ca^{2}$ chlorides into isolated living adductor magnus muscle fibers of the frog. M/5000 $CaCl_2$ showed a 50% shortening of the muscle fiber. Isotonic $MgCl_2$ produced relaxation. The injection volume was 10% of the muscle fiber volume. This research brought Heilbrunn's insight about calcium into focus. In response to this work, Heilbrunn and Wiercinski opposed Szent-Györgyi's notion that potassium ion was the primary ion.

A. V. Hill and Albert Szent-Györgyi caused the "hush of neglect." Hill thought of diffusion in a solid cylinder, before the discovery of the sarcoplasmic reticulum. Szent-Györgyi applied ATP and $K^+$ ion in minced muscle to determine if this was the primary stimulus for muscle contraction. He made sure that the muscle preparation he used was "dead" in contrast to Heilbrunn and Wiercinski's work with freshly dissected muscle fibers in calcium-free media.

Stoltz and Bereiter-Hahn (1987, 1988) injected calcium by iontophoresis to observe sequestration in live endothelial cells in tissue culture.

*Colloid chemistry of protoplasm*

Heilbrunn (1928–1932) performed cutting experiments with *Stentor* and *Amoeba* and showed that the "naked protoplasm" quickly formed a film in calcium-containing solution. From these observations he postulated colloid chemistry as the basis of protoplasmic activity. He believed that protoplasmic activity is transmitted from one part of a cell to another or from one cell to another. The reaction has three stages: (a) free calcium is liberated, (b) calcium unites with other materials to form a thrombin-like substance which, in the sea urchin, is named ovothrombin, and (c) a reaction occurs between this thrombin-like substance and another substance to form a precipitation product. He also found that ovothrombin is surface active at the rate of 25 cm/s, and that this is a self-propagating reaction.

*Surface precipitation reaction*

Heilbrunn (1928–1932) also described the surface precipitation reaction. He noted that the reaction is retarded or inhibited by low temperature, that calcium is essential, and that pigment granules play a role in the sea urchin egg. He also found that calcium is released in the cell interior, that calcium reacts with pigment granules or a constituent named ovothrombin, and that ovothrombin reacts with substances (sea urchin egg) in the protoplasm—presumably a protein—to cause vacuole formation. He also discusses the action of acids and alkalies.

*Neurobiology: theory of function and evolution*

Kretzinger (1979) showed that many basic cellular mechanisms are common to all eukaryotic cells, are of common origin, and are related in evolution.

Campbell (1983) showed that calcium plays a role in nerve terminals, neurohormones, neuromuscular junc-

tions, neurones, neurophysin, neurosecretions, neurotransmitters, and receptors.

Mandel and Eaton (1987) and Snutch *et al.* (1986) characterized voltage gated calcium channels in *Xenopus* oocytes after the injection of RNA obtained from electrically excitable tissues. Mandel and Eaton (1987) and Chad *et al.* (1986) also showed that phosphorylation maintains calcium channel activity in dialyzed molluscan neurons.

Five postulates have been suggested for the intracellular function in eukaryote cells.

(1) All resting eukaryotic cells maintain a concentration of free $Ca^{2+}$ in the cytosol between $10^{-7}$ *M* and $10^{-8}$ *M*.

(2) The sole function of $Ca^{2+}$ within the cytosol is to transmit information through binding to a specific receptor.

(3) The target of $Ca^{2+}$, functioning as a second messenger, is a protein in the cytosol.

(4) Calcium modulated proteins contain "E.F. hands" to maintain conformation (Krietzinger, 1978).

(5) Cells initially extruded calcium so they could use phosphate as their basic energy currency; $Ca_3(PO_4)_2$ is insoluble.

*Comparison of concepts*

Comparing Heilbrunn's (1928) work on the colloid chemistry of protoplasm with Kretzinger's (1979) work on the theory of the function and evolution of calcium, we find that each suggested five postulates about the function of calcium. Chance (1968) presented six postulates.

*Homeostasis*

Heilbrunn's general physiology textbooks (1938, 1943, 1952) do not mention homeostasis. However, he does discuss the topic in his 1956 text *The Dynamics of Living Protoplasm.*

Rasmussen and DeLuce (1963) reviewed calcium regulation extensively. They mention (p. 147) isolated mitochondria in calcium and phosphate exchange as a new approach to calcium.

Mathews *et al.* (1981) discuss the role of calcium in the organism as a reciprocal relationship between intracellular and extracellular functions. Currently, it is believed that the intracellular ionic concentration is $10^{-7}$ *M* and the extracellular is $10^{-3}$ *M*. A proper ionic intracellular calcium level is maintained by the mitochondria (p. 424). In bone mineralization, large amounts of calcium and phosphate are utilized.

The general physiological effects of calcium can activate or control basic physiological processes, such as irritability, contractility, conduction, secretion, mitosis, reproduction, and integration. The role of calcium in special nerve organs, other tissues, and cell types is being investigated.

McGrath *et al.* (1986) showed that active transport through the bone membrane appears to be involved in the regulation of calcium levels in the blood stream. The present development limits the possible active transport mechanisms for the mineral dissolution/deposition to an outward-directed pump for the hydrogen ion, an outward-directed pump for the phosphate ion, or an inward-directed pump for the hydrogen ion.

Stoltz and Bereiter-Hahn's (1987) observations support the assumption of two sequestration mechanisms: (1) a high affinity for $Ca^{2+}$ capacity and a low affinity for $Ca^{2+}$ ($5 \times 10^{-15}$ $mols^{-1}$) and (2) a low affinity for $Ca^{2+}$ and a high capacity ($10–40 \times 10^{-15}$ $mols^{-1}$) for calcium accumulation. These mechanisms are saturable. The high affinity $Ca^{2+}$ compartment is the endoplasmic reticulum, and the low affinity system is the mitochondrion. There is a five- to eight-fold higher $Ca^{2+}$ sequestration capacity, and the velocity of sequestration is equal in both cell types (XTH2 and primary cells).

*Calcium homeostasis*

Claude Bernard (1878) proposed constancy of the internal environment of animals and plants. Subsequently, biologists have made a considerable effort to understand the regulation of this internal environment. The endocrine systems, the parathyroid gland, vitamin D-hormone, modern-day calcitonin, phosphate (ATP), and the mitochondria play roles in the integrated organic scheme.

Cannon (1932) postulated that homeostasis controls the internal environment of warm-blooded animals. Oxygen, pH, salts, food (glucose, amino acids, lipids), proteins, and blood concentrations are maintained within narrow limits. However, Richards (1953) criticised homeostasis. He claimed that in chronic disease, homeostasis does not prevail in all functional bodily processes.

Rasmussen and DeLuca (1963) discussed the parathyroid gland's role as an essential calcium control system in the mammal. In evolution the parathyroids first appeared in the amphibia. Teleosts all possess a calcified endoskeleton. Salmon, in their migratory passage from the sea to fresh water, achieved calcium homeostasis in spite of the dramatic fluctuations in the calcium content of the external environment. This happens without parathyroid glands. It is suggested that the role of the D vitamins in calcium homeostasis is older in evolution than the parathyroids. Calcium, phosphate, and vitamin D are essential in the diets of all higher animals.

In summary (pp. 164–165), the parathyroid hormone, vitamin D, and phosphate function in the cellular accu-

mulation and transport of calcium in a variety of tissues. Vitamin D regulates the activity of calcium-specific systems in cellular and mitochondrial membranes. The ability of cells in epithelium to bring about the net transfer of ions, as distinct from ion accumulation, is related to structural characteristics and polarity.

Bianchi (1968) wrote that in mammals, calcium and phosphate are controlled by parathyroid hormones, vitamin D, and thyrocalcitonin in intracellular and extracellular function. Over-secretion of parathyroid results in osteitis fibrosa cystica, absence of vitamin D causes rickets in the young and osteomalacia in the adult, and overproduction of thyrocalcitonin may be related to osteoporosis. The underlying causes of disturbances in phosphate and calcium metabolism affect calcium transport across cell membranes and intracellular organelles.

*Protein kinase and protein kinase C*

A $Ca^{2+}$ phospholipid-dependent protein kinase is recognized as a pivotal regulatory element in signal transduction, tumor promotion, cell regulation and rat brain cytosol (see below).

Protein kinase and protein kinase C are reported in *Methods in Enzymology* (1987) and by Mandel and Eaton (1987) (see also Chad *et al.*, 1987, for protein kinase, and Strong *et al.*, 1987, for protein kinase C).

Several methods have described protein kinases (phosphoproteins) from bovine heart, pig spleen, rat brain, rabbit renal cortex, and rabbit brain. When assessed with a 7–9% acrylamide gel, it is tentatively identified as having an "apparent" molecular weight of 80,000. "On 18% acrylamide gel, it migrates with an apparent molecular weight of about 120,000" (p. 419, *Methods in Enzymology*).

*The calcium paradox and the multifaceted behavior of the calcium ion*

Durrier and Meijler (1983) wrote that the calcium paradox is only one aspect of the multifaceted behavior of the calcium ion in all living material, but together in its role in the myocardial excitation complex it is a very important one. One of the most important spin-offs of the study of the effects of calcium on living tissue is the discovery of calcium antagonists (Fleckenstein, 1977).

As early as 1920 (see Heilbrunn, 1937, p. 348), there is a literature on "paradoxes." These include a potassium paradox, a calcium paradox, and a thermo paradox. Can these occur in the metastable state of calcium (Pauling and Goudsmit, 1930)?

The future of the calcium paradox model—for myocardial, ischaemic, and pre-perfusion damage—depends on the development of compounds that may protect the myocardium.

*Anghileri and Anghileri (1982)*

Volume I discusses the chemistry of calcium and gives both the theoretical and practical basis to interpret its role in the function of normal and pathological biological systems. The precipitation process of hydroxyapatite (HAP) as it relates to bone and tooth is reviewed in detail. Mathematical models for analyzing calcium kinetics are discussed.

Volume II reviews the role of calcium in membrane stability and the regulation of various membrane functions. It describes basic properties of the mechanisms for calcium inflow and outflow across the plasma membrane. It also assesses the results of investigations of plasma membrane, calcium movement in other tissues and summarizes the effects of hormones, neurotransmitters, other cell stimuli, and cell injury on these processes.

Volume III examines the role of calcium in the insulin release process, in taste and olfactory functions, and in dentinogenesis.

*Calcium out of control*

Poole-Wilson *et al.* (1984) wrote that calcium is invasive in myocardial cell necrosis following severe hypoxia or ischaemia. The mechanisms are: (1) lack of energy, loss of enzyme systems, (2) mechanical effects, damage of the structural matrix of the cell, and (3) membrane damage in the mitochondria and sarcolemma. The common pathway is cell necrosis, and calcium "overloads" the cell. A causal mechanism is the accumulation of calcium during myocardial hypoxia or ischaemia followed by reoxygenation or pre-perfusion related to the development of cell necrosis.

*Pools*

The idea of "pools" for calcium and other ions derives from the observations of food vacuoles in protozoa. Protozoa, such as *Amoeba*, take in food particles at the edge of the plasmalemma forming a food vacuole. Undigested particles are subsequently expelled by the food vacuole. *Paramecium* has a gullet for taking in food particles (endocytosis) and smaller protozoans. The undigested portion is taken into a contractile vacuole that is expelled (exocytosis) through the micropyle of the cuticle or cortex.

Heilbrunn (1937) suggested that bound calcium exists in the living cell. For example, calcium is bound in the cortex of the cell. Stimulation from a wide range of phenomena could elicit the release of calcium from the cortex—a pool. A 10 $\mu$m cell, as suggested by Brown (1984), is the typical size of cells in many tissues. The cortex could easily hold a pool of 3000 calcium ions. Permease could release this calcium to the cytosol. This would be

in equilibrium with the calcium concentration of $10^{-7}$ to $10^{-8}$.

How many calcium ions enter a living cell under physiological conditions? The calcium ion has an invasive action. When present in the cytosol in any amount greater than $10^{-7}$ to $10^{-8}$, it causes breakdown of cellular function. Brown (1984) considered a spherical cell of 10 $\mu$m diameter. How many calcium ions would a cell this size require? Considering the concept of calcium pools in the cytosol of small cells (10 $\mu$m diameter), how many calcium ions would be present in a pool? The intracellular environment and anatomy of the cell should be considered. Brown considered $10^{-8}$ intracellular calcium activity: "The cell would contain about 3000 free Ca ions." Where are these Ca ions located in the cell? How many would be used during and following an "action potential"? What would be the number of Ca ions in a pool? Also, how many pools exist in the cytosol of a small cell? Have "pools" been detected as morphological entities? Porter (1961) found the endoplasmic reticulum, which extends from the cell membrane to the nucleus around the mitochondria and the Golgi apparatus.

At least three pools have been found in the cytosol of secretory cells (Campbell, 1983, p. 329). The cortex or cell membrane would be the site from which calcium ions can enter the cytosol to be stored in a pool (endocytosis). An event such as an action potential elicited by a stimulus would release calcium (exocytosis).

Campbell (1983, p. 224) writes that intracellular calcium can be altered by several mechanisms: (1) an effect on the permeability of the cell membrane, (2) An effect on the intracellular store, and (3) an effect on the calcium pump.

An acceptor protein with a chemotactic property would be a cyclic AMP protein kinase. *In vitro* studies have shown soluble and insoluble membrane-bound proteins.

There are calcium channels in the cortex as well as an endoplasmic reticulum in the cytoplasm. The molecular mechanism of intracellular calcium is as a regulator.

### *Calcium antagonists*

A group of organic drugs block the entrance of calcium into living cells. The invasive property of calcium is to enter damaged cells. The surface precipitation reaction is a cell's response to injury.

In heart disease the heart cells could be damaged and the adverse action of calcium would cause the cell to disintegrate. But by preventing calcium from entering the cell, can it repair itself?

The classical experiment of crushing a cell under a coverslip on a microscope slide in a calcium environment produces the surface precipitation reaction. Cytosolic calcium keeps the nucleus and the cellular organelles (ribosomes, liposomes, mitochondria, etc.) in an intact membrane.

Fleckenstein *et al.* (1966/1967) coined the term "calcium antagonist." Godfraind and Kaba (1969) showed inhibition of calcium by cinnarizine and chlorpromazine of contraction in vascular smooth muscle. Nichols and Wasserman (1971) compared external and internal exchange of calcium in isolated cells. Rahwan and Witiak (1982) wrote that calcium entry into cells depends on the diversity of structure in multiple modes of action and sites of action.

Naylor and Dillon (1986) wrote:

"Ca antagonists are a novel group of drugs useful in management of a variety of cardiac disorders. They differ from one another in terms of their chemistry, tissue specificity and selectivity. As a group, however, they share the common property of slowing $Ca^{2+}$ entry through voltage-activated, ion-selective channels. Some of them exhibit other properties, including that of interfering with $Na^{+}$ transport. At least one of them, diltiazem, has an intracellular action. Specific high and low affinity binding sites have been identified for two of the major groups of $Ca^{2+}$ antagonists, with the binding sites for verapamil and its derivatives being distinct from those which can be occupied by the dihydropyridines. The number ($B_{max}$) and affinity ($K_D$) of these binding sites changes under certain pathological conditions—including a reduction in ischaemia and in spontaneous hypertension, and increase in the latter, at present, only demonstrated for the dihydrpyridine binding sites. The sensitivity of a particular tissue to these drugs will depend upon a number of factors including the number of binding sites that are present, the contribution made by the $Ca^{2+}$ entering tissue, and properties which are peculiar to a particular type of verapamil, they exhibit use-dependence."

Moser (1986) discusses calcium entry blockers in a historical perspective on the management of hypertension. These drugs lower blood pressure by reducing peripheral resistance through the vasodilation of the blood vessels and capillaries in blocking calcium that acts as a powerful vasoconstrictor.

### *Calcium homeostasis control*

In their volume on calcium antagonists, Van houtte *et al.* (1988) include many papers to define the existence of various types of calcium channels. They write:

"The clinical perspective is represented by the most recent cardiovascular, renal, gynecological, endocrinological and neurological applications of calcium antagonism research; the ever widening circle of disorders to which calcium antagonists are being applied now include heart failure, hypertension, stroke, migraine headaches,

epilepsy, vertigo, dysmenorrhea, and certain disfunctions of the kidney and liver."

Historical reviews and a broad survey of current research define new directions for future research. For example, work pointing toward the treatment of long-term diseases, particularly atherosclerosis, is discussed, and data are presented that indicate that some calcium antagonists enhance low-density lipoprotein uptake by increasing the number of low-density lipoprotein receptors. These data, which indicate that calcium antagonists could be active at the gene level and thus affect mRNA and protein synthesis, suggest entirely new approaches to drug research.

The possibility that calcium antagonists influence the central nervous system is explored. Researchers look forward to neural applications, such as protecting the brain against ischaemia and anoxia, that could prove as beneficial as the cardiovascular application.

The events triggered by exaggerated calcium influx and release clarifies how the harm caused by these events, which undoubtedly contribute to cell death, have prompted scientists to attempt the control of calcium homeostasis by reducing excessive calcium influx, facilitating intracellular calcium buffering, and counteracting calcium-evoked reactions.

*Conceptual advances*

Examination of current calcium literature indicates that there has been little, if any, inquiry into the molecular mechanisms that no doubt are involved in calcium at the atomic level. Dr. Robert Silver (Cornell University, pers. comm.) is observing one atom in the cytosol of the sand dollar egg using video microscopy techniques.

Conceptual advances in cellular recognition elements have recently been made involving cell receptors. Fourteen types of receptors that are specific for biological molecules in the cell have been recognized.

Putney (1986) and Peterson (1987) suggest a model for receptor-regulated calcium entry. Five previous hypotheses are reviewed. Putney's proposed model is termed "capacitative $Ca^{2+}$ entry."

Clearly, all living cells are born with the inherent electrical property of life. Calcium does not "work" in the biological sense in non-living cells.

*Unanswered questions*

Much has been written regarding calcium's entry into and out of the living cell and its role in cell death. Recently developed techniques used by calcium researchers have opened up a vista of complex biochemical reactions.

Calcium is essential for life. It is present in plants and in the blood of animals, and it is used, as needed, in cellular functions. But what happens in the living cell? How many things can happen or be contained in one cell? Finally, what is the role of calcium in the living organism both on earth and in outer space?

## Summary

After examining the extensive chronology about calcium, I have attempted to discuss the fundamental implications of calcium in a reciprocal role in maintaining the extracellular and intracellular functions in biological systems. Homeostasis is maintained in the living organism by numerous mechanisms, but this is not so in the diseased state.

The receptor protein calmodulin, protein kinase, protein kinase C, and a number of other proteins have been described. The calcium paradox found in cardiac muscle function remains. Why calcium?

The question, as yet, goes unanswered. No other substance is capable of maintaining, with the cooperativity of organismic systems, normal homeostasis while performing biological, building, and physiological functions.

In summary, this paper is merely an overview—a small piece of the much larger, complex, and ever-changing puzzle that is calcium.

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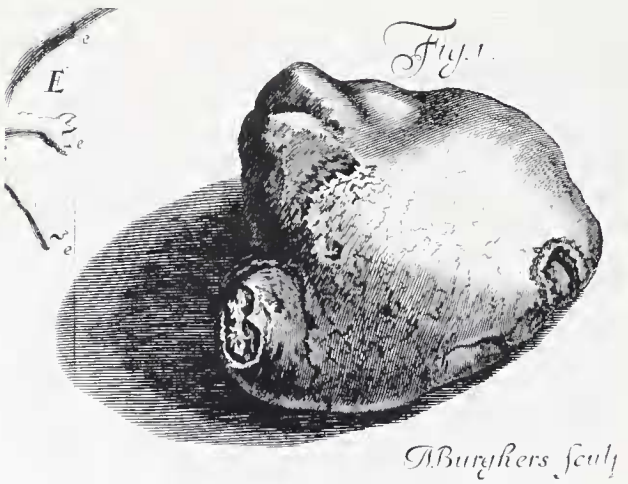
**Appendix**

## *A* Poſtſcript *to the* Publiſher, *containing a ſhort account of two* Human Calculi *of unuſual form and bigneſs, from the ſame* F. S. M. D.

*SIR,*

I Here ſend you the Figure of a *Stone*, *Fig.* 1. of a prodigious *ſize* and as rare a *ſhape*, ſomewhat reſembling indeed the *Kidney*, for that was quite worn away, and this *ſtone* fill'd up the *place*, it weigh'd indeed ſomewhat more when I took it out of the Body, than it does now, for it then weigh'd ſeven ounces and a half; there is no *Hiſtory* that relates any account of a *ſtone* generated in the *Kidney*, that does near parallel this. Without breaking it aſunder, I can find it does conſiſt of ſeveral *laminæ* laid over one another, as that of the *Bladder* does: I took the *Circumference*, and found it to meaſure ſeven Inches upon the round.

That taken out of the Body of the late *Duke* of *Norfolk's* Father, *Fig.* 2. was brought not long ſince to the *R. Society* by Sr. *Theodore de Vaux*, who gave me leave to ſend you the *Figure* of it, which you ſee is branched, and ſeems to have ſpread ſome branches into great *Veſſels*, whether *Arteries*, *Veins* or into the *Ureter* I cannot determine, tho theſe as well as the *Pelvis* ſeem to have been fill'd up by this great *ſtone*: yet this comes far ſhort of that before mention'd, ſince it *weighs* but four ounces and a half: a *ſtone* indeed of an incredible ſize to be found in the *Kidney*. The meaſure longwiſe from one extreme to the other made four Inches compleat: the extenſion of the *Branches* from one to the other meaſur'd croſwiſe or tranſverſly, 3 Inches and a half. This is deſervedly laid up in the *Repoſitory* of the *Royal Society*, as a great but ſorrowful *Rarity*, having cauſed the death of ſo great a *Patron* of *Learning*.




From Slare (1693).